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IMMEDIATE BREAST RECONSTRUCTION: PATIENT INFORMATION, REGIONAL VARIATIONS AND EFFECTS OF RADIOTHERAPY

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Immediate breast reconstruction: patient information, regional variations and effects of radiotherapy

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ABSTRACT

The type of surgical treatment in breast cancer shows strong regional variation. The aim of studies I-III was to specifically investigate causes of the disparity in breast conservation and immediate breast reconstruction (IBR) rates in the six Swedish healthcare regions. All women who underwent any surgical intervention for primary breast cancer in Sweden in 2013 were included. Tumour and treatment data were retrieved from the Swedish National Breast Cancer Register, and socioeconomic background data from the Central Bureau of Statistics Sweden. Postal questionnaires regarding preoperative information and involvement in the preoperative decision-making process were sent to all mastectomy patients, with a response rate of 76.3%. Of 7735 women, 4604 (59.5%) had breast conservation and 3131 (40.5%) mastectomy, 267 of whom also received IBR. While tumour and patient characteristics predictably affected surgical treatment, they could not explain regional variations. Higher socioeconomic status resulted in a higher rate of IBR and breast conservation. Patient-reported preoperative information (OR 12.73, 95% Cl 6.03-26.89) and involvement in the decision-making process (OR 2.56, 95% Cl 1.14-5.76) remained strong independent predictors of IBR even after adjustment for socioeconomic factors.

After implant-based IBR, primary expander devices are frequently exchanged for permanent silicone implants. The aim of study IV was to assess whether the timing of this exchange procedure in relation to the completion of post-mastectomy radiotherapy (PMRT) and other clinical factors affect surgical complications rates resulting in implant failure (primary outcome). All women previously treated with an IBR who underwent implant exchange and/or capsulectomy at Karolinska University Hospital between 2005 and 2015 were included. Detailed information was collected through individual medical chart review. The final cohort consisted of 475 breast cancer patients with 707 implant revision surgeries in 542 breasts, in which 33 cases of implant failure were observed. PMRT, smoking and diabetes were confirmed as risk factors, while time from completion of PMRT to revision surgery was not associated with the outcome. Additional risk factors were a previous axillary lymph node dissection and a history of a post-IBR infection.

A common problem in implant-based IBR is the development of a capsular contracture secondary to PMRT. Study V evaluated gene expression patterns in biopsies from irradiated (n=13) and non-irradiated (n=12) capsular tissue harvested during implant exchange surgery in IBR patients, and gene expression levels were compared in order to identify the most differentially regulated genes in order to explore the underlying biology with the wider aim to generate knowledge enabling the development of therapeutic strategies. Radio-responsive genes were most often involved in inflammation immune response, and both innate and adaptive immune system responses were confirmed by immunohistochemistry on the protein level. There was a significant upregulation in CD20+ B-cell counts in irradiated biopsies, which was supported by gene expression analysis. However, the findings need to be confirmed in a larger study.

POPULÄRVETENSKAPLIG SAMMANFATTNING

Den primära behandlingen av bröstcancer är i de flesta fall kirurgi. Vilken typ av kirurgisk behandling som patienterna erhåller varierar stort mellan de olika sjukvårdsregionerna. I studierna I-III var syftet att studera orsakerna till skillnaderna i frekvens av bröstbevarande kirurgi (BCS) och omedelbar rekonstruktion (IBR). Alla kvinnor som opererades för bröstcancer år 2013 identifierades från Nationella kvalitetsregistret för bröstcancer. Tumördata och behandlingsdata rekvirerades och kopplades till socioekonomiska bakgrundsdata från Statistiska centralbyrån. Ett frågeformulär skickades till alla patienter som genomgått en mastektomi avseende vilken grad av preoperativ information man erhållit om olika kirurgiska behandlings- och rekonstruktionsmetoder och i vilken grad man känt sig delaktig i den preoperativa beslutsprocessen. Svarsfrekvensen på enkäten var 76,3 %. Av totalt 7735 kvinnor med bröstcancer hade 4604 (59,5 %) opererats med BCS och 3131 (40,5 %) med mastektomi, varav 267 också genomgick IBR. Tumörstatus påverkade valet av kirurgisk behandling men kunde inte förklara de regionala variationerna, däremot fanns det signifikanta skillnader av preoperativ information och delaktighet mellan de olika regionerna. Högre socioekonomisk status resulterade i en högre frekvens av både omedelbar rekonstruktion och bröstbevarande kirurgi. Även efter justering för socioekonomiska faktorer förblev patientrapporterad preoperativ information och delaktighet i beslutsprocessen starka oberoende prediktorer för IBR.

Oftast innebär en direkt rekonstruktion med implantat flera rekonstruktiva steg. Efter implantatbaserad IBR, där man vanligast opererar in en protes som kan expanderas, kan denna senare bytas till ett permanent silikonimplantat. Om strålbehandling ges mot ett bröst rekonstruerat med implantat innebär det risk för kapselkontraktur som innebär att den naturliga kapseln runt protesen blir kraftigare och drar ihop sig, vilket leder till kosmetisk försämring men ibland också till smärta. Syftet med studie IV var att bedöma den optimala tidpunkten för ett protesbyte eller annan revisionskirurgi i själva proteshålan efter avslutad strålbehandling samt att studera ytterligare kliniska riskfaktorer som påverkar komplikationsfrekvensen, och som kan resultera i att protesen behöver avlägsnas. Alla kvinnor som tidigare behandlats för bröstcancer med en mastektomi och IBR och sedan genomgick protesbyte och/eller klyvning av proteskapseln vid Karolinska Universitetssjukhuset mellan 2005 och 2015 inkluderades. Genom individuell journalgranskning samlades detaljerad information. Kohorten bestod av 475 kvinnor med 707 revisionsoperationer i 542 bröst. Protesen behövde avlägsnas på grund av postoperativa komplikationer i 33 fall. Strålning mot det rekonstruerade bröstet efter mastektomi (PMRT), rökning och diabetes bekräftades som riskfaktorer för protesförlust, medan tiden från avslutad PMRT till revisionsoperation inte var relaterat till protesförlust. Ytterligare riskfaktorer var om patienten genomgått lymfkörtelutrymning vid IBR och om patienten haft en postoperativ infektion i samband med den tidigare IBR operationen.

Som beskrivits ovan är utvecklingen av kapselkontraktur ett vanligt problem vid implantat-baserad direktrekonstruktion då strålbehandlingen ges postoperativt. För att bättre kunna förstå biologin bakom utvecklingen av kapselkontraktur utvärderades i studie V genuttrycksmönster i biopsier från strålade (n=13) och icke-strålade (n=12) kapselvävnader efter IBR. Dessa biopsier samlades in vid operationer där implantatet byttes ut efter tidigare IBR. Man jämförde skillnader i genuttryck mellan de båda grupperna för att identifiera de gener som påverkades mest av strålningen och fann att många av dessa var involverade i flera inflammatoriska processer. Många av de gener som påverkades mest av strålning visade sig vara involverade i både det innata och adaptiva immunförsvaret många år efter strålbehandling. Immunohistokemisk färgning av inflammatoriska celler gav ytterligare stöd för detta. Särskilt tydligt var en ökad förekomst av i CD20 + B-celler i bestrålade biopsier, vilket är intressant mot bakgrund av genuttrycksanalyser som visade B-cells medierat immunsvar som ett av de tydligaste resultaten.

LIST OF SCIENTIFIC PAPERS

I. **National study of the impact of patient information and involvement in decision-making on immediate breast reconstruction rates**

   Frisell A, Lagergren J, de Boniface J.

   Br. J. Surg., vol. 103, no. 12, pp. 1640–1648, Nov. 2016

II. **Influence of socioeconomic status on immediate breast reconstruction rate, patient information and involvement in surgical decision-making**

   Frisell A, Lagergren J, Halle M, Boniface J.

   BJS Open, Jan. 2020.

III. **Socioeconomic status differs between breast cancer patients treated with mastectomy and breast conservation, and affects patient-reported preoperative information**

   Frisell A, Lagergren J, Halle M, Boniface J.

   Breast Cancer Res Treat, vol. 179, no. 3, pp. 0167-6806, Jan. 2020.

IV. **Risk factors for implant failure following revision surgery in breast cancer patients with a previous immediate implant-based breast reconstruction**

   Frisell A, Lagergren J, Halle M, Boniface J.

   Submitted for publication.

V. **Capsular inflammation after immediate breast reconstruction – an exploratory study comparing gene expression patterns and inflammatory cell infiltration in irradiated and non-irradiated breasts**

   Frisell A, Bergman O, Kahn A, Gisterå A, Fisher R, Lagergren J, de Boniface J, Halle M.

   Manuscript.

CONTENTS

LIST OF ABBREVIATIONS

AI	Aromatase inhibitor
ACT	Adjuvant chemotherapy
ALND	Axillary lymph node dissection
BCT	Breast-conserving therapy
BCS	Breast-conserving surgery
CI	Confidence Interval
CMF	Cyclophosphamide, methotrexate, fluorouracil
DBR	Delayed breast reconstruction
DIEP	Deep Inferior Epigastric Perforator
EBCTCG	Early Breast Cancer Trialists' Collaborative Group
HER2	Human epidermal growth factor receptor 2
IBR	Immediate breast reconstruction
NACT	Neoadjuvant chemotherapy
NKBC	Swedish National Breast Cancer Register
OR	Odds ratio
RT	Radiotherapy
SNB	Sentinel node biopsy
PMRT	Post-mastectomy radiotherapy
RR	Relative risk

1 LITERATURE REVIEW

1.1 Breast cancer

Globally, breast cancer ranks as the second most diagnosed cancer, and more than 1.7 million new cases were registered worldwide in 2015. Breast cancer accounts for 10% of global cancer cases and for 22% of all cancer among women. Incidence rates are higher in developed countries with about 80 cases per 100,000 women and lower in less developed countries with <40 cases per 100,000 women (1). Further, they are on the rise in emerging economies, probably owing to a more “western-like” lifestyle but also to improved diagnostics (1). Since the last century, some decrease in the incidence in developed countries has been seen, which might be the result of a declining use of hormone replacement therapy (HRT) (2) and of adherence to mammography screening (3). In Sweden, 8400 women were diagnosed with breast cancer in 2012, 8630 in 2015, and 8463 in 2016 (4). Survival has been increasing in all Western countries during the last three decades. Better survival is attributed to the implementation of mammography screening, advancements in adjuvant and neoadjuvant treatments such as chemotherapy, endocrine and targeted therapies, to better general healthcare, an increasing degree of self-examination, and breast cancer awareness (5).

The golden standard for diagnosing breast cancer is triple assessment, which includes physical examination, imaging by mammography and/or ultrasound, and either fine-needle aspiration cytology or needle biopsy. This diagnostic combination has a high sensitivity and reaches almost 100% diagnostic accuracy (6). All breast cancer patients should be discussed at a multidisciplinary team conference both pre- and postoperatively according to Swedish guidelines. Such conferences were established in Sweden more than 25 years ago and bring together a breast surgeon, breast oncologist, breast radiologist, pathologist, breast nurse and sometimes a plastic surgeon. Based on comprehensive diagnostics and team discussion, a treatment recommendation is given. Treatment can include surgery, radiotherapy, chemotherapy, endocrine treatment as well as targeted therapies.

1.1.1 Surgical treatment

William Halsted coined the term “radical mastectomy” in 1892, including removal of all breast tissue, overlying skin, pectoral major and minor muscles and axillary lymph nodes (7). In 1948, the operation was modified in order to decrease postoperative morbidity and disfigurement by preserving the pectoral major muscle, a procedure known as modified radical mastectomy (8). Still, while the Halstedian paradigm stated that the cancer would only spread per continuum along lymphatics, and more radical resections would thus lead to better cure, extensive surgery did not decrease mortality. The work by Fisher et al resulted in the notion that breast cancer is rather a systemic than a local disease, a concept still valid today (9). Subsequently, as a combined result of better understanding of the biology and behaviour of tumours, as well as earlier detection and thus smaller tumour sizes, breast-conserving surgery, where only the tumour-bearing part of the breast is removed with an appropriate margin, was introduced in the 1970s. In randomized multicentre trials with long-term follow-up, breast-conserving surgery with subsequent whole-breast radiotherapy (together termed breast-conserving therapy, BCT) achieved survival rates comparable to mastectomy despite higher local recurrence rates at that time (10,11). Breast-conserving therapy has become the golden standard for women with early-stage breast cancer and is today performed in approximately 83% of women with a breast cancer up to 3 cm in size (4). Several retrospective studies have shown BCT to be oncologically at least equivalent to mastectomy, if not better (12–14). Mastectomy, however, is still indicated for larger or inflammatory tumours, in tumour multifocality, as a result of patient choice, or in case of an unfavourably high ratio of tumour volume to breast volume, and is registered as the final surgical intervention in up to 40% of cases in Sweden with some regional differences (4).

In axillary surgery, there has been a major paradigm shift from viewing it as a therapeutic procedure to it being a predominantly diagnostic measure. Sentinel node biopsy (SNB) has in many situations replaced the more extensive axillary lymph node dissection (ALND), removing 10 lymph nodes or more. The sentinel node, introduced in breast cancer surgery in 1994 by Giuliano et al and Krag et al (15,16), is the first node that drains the tumour bed and is thought to reliably represent the status of the remaining axilla. Long-term follow-up studies confirm that SNB alone is safe as a staging method in patients with clinically node-negative breast cancer, implying that an ALND can be omitted in case of a negative SNB without any impact on axillary recurrence rates (16–18). The use of a SNB instead of an ALND leads to decreased arm morbidity since fewer lymph nodes are removed (19,20). With improved systemic therapies and decreasing recurrence rates, the need for ALND has been questioned even after a positive SNB: The ACOSOG Z0011 and IBCSG 23-01 trials showed no difference in survival or axillary recurrence rate regardless of whether an ALND was performed or not (21,22). Only breast-conserving surgery was included and thus, data on mastectomy and omission of ALND are lacking (23). Likewise, it has been shown that axillary radiotherapy can safely replace ALND, but even here, mastectomy patients are under-represented (24). An ongoing Swedish randomized trial (SENOMAC) includes 3500 patients in several European countries in order to assess the need of ALND in SN-positive cases, including those operated with a mastectomy (25).

1.1.2 Chemotherapy

Chemotherapy has been used as breast cancer treatment since about 1960; the first chemotherapy showing a survival benefit was a combination of cyclophosphamide, methotrexate and fluorouracil (CMF) (26–28). The second generation of chemotherapy, containing the anthracyclines doxyrubicin and epirubicin, resulted in further survival improvements when used in combination with CMF. A meta-analysis by the Early Breast Cancer Trialists' Collaborative Group (EBCTCG) assessing data of 100 000 women showed a significant decrease in 10-year breast cancer mortality compared to no cytotoxic treatment (21.5% vs. 27.6%, RR 0.76 [0.68–0.84]) (27). Furthermore, anthracycline-based regimes yield a larger decrease in 10-year breast cancer mortality than CMF (24.1% vs. 20.0%, RR 0.80 [0.72–0.88]), and the combination of 5-fluorouracil, epirubicin and cyclophosphamide reduced local recurrence rates by about 16% and mortality by 21% (27). Later, the addition of taxanes to anthracycline-based regimes further improved 5-year breast cancer mortality from 11.5% to 10.1% (RR 0.88 [0.81–0.95]) (29). Chemotherapy is most commonly given as adjuvant (postoperative) treatment (ACT), but neoadjuvant (preoperative) treatment (NACT) is an increasingly used option. NACT was initially used only in large inoperable or inflammatory breast cancer but today even tumours as small as 2 cm but with aggressive tumour biology, such as HER2-positive and triple-negative cancers, are considered for NACT. NACT has the potential advantage to be tailored according to the individual tumour response monitored in real time, and opens up possibilities for accelerated drug development and approval. In a meta-analysis by the EBCTCG, assessing data from 4756 women with early breast cancer from ten randomized trials, NACT was shown to achieve a 15-year breast cancer mortality equal to ACT (34.4% vs 33.7%; 1.06 [0.95–1.18]), but resulted in a higher frequency of BCT (64% vs. 49%) as well as a higher local recurrence rate (30). In Sweden, 11.2% of all breast cancer was treated with NACT in 2017 (4). General indications for chemotherapy are high grade and/or high proliferation, triple-negative or HER2-positive subtypes, lymph node positivity and young age. Chemotherapy, whether given pre- or postoperatively, has significant side effects, and biomarkers or genetic profiling predicting response may give us a possibility to customize chemotherapy to avoid overtreatment.

1.1.3 Endocrine therapy

The vast majority of breast cancers (about 80%) have receptors for oestrogen (ER), i.e. they are oestrogen sensitive. Either by blocking the receptors or by lowering circulating hormone levels, adjuvant endocrine therapy reduces the risk of recurrence and even has a prophylactic effect on the contralateral breast. The first endocrine drug, Tamoxifen, is a selective oestrogen receptor modulator blocking ER by competing with oestrogen (31). Five years of treatment with Tamoxifen reduces recurrence rates by nearly 50% (RR 0.53 during the first four years and RR 0.68

during years 5–9) and breast cancer-specific mortality by one third during the first 15 years after starting treatment (RR 0.71 during years 0–4, 0.66 during years 5–9, and 0.68 during years 10–14) (32). Aromatase inhibitors (AIs) such as anastrozole are non-steroidal inhibitors of the aromatase enzyme, resulting in a decreased conversion of precursor stages to oestrogen. AIs are commonly indicated in medium to high-risk breast cancer in postmenopausal women, and further decrease both 10-year recurrence rates and 10-year mortality (RR 0.85, $p<0.01$) when compared with Tamoxifen (33). A publication from EBCTCG in 2017 showed that after five years of adjuvant endocrine therapy, breast cancer recurrences continue to occur throughout a follow-up period of 5 to 20 years. The risk of late distant recurrence was strongly correlated with larger tumour size and more advanced axillary status, and accordingly, prolonged endocrine treatment for 10-15 years is recommended in selected risk groups today (34).

1.1.4 Targeted therapy

Human epidermal growth factor receptor 2 (HER2) is a tyrosine kinase receptor located on the cell surface. The HER2 gene is expressed in about 15-20% of all breast cancers, and is associated with more aggressive tumour biology and poor prognosis. It is, however, also a predictive biomarker for therapeutic response (35): trastuzumab is a monoclonal antibody binding to the HER2 receptor, thereby inhibiting cell proliferation. Together with chemotherapy, trastuzumab leads to a 34% relative improvement in overall survival in HER2-positive breast cancer patients (HR 0.66; [0.57-0.77]) (36). In the neoadjuvant setting, a "double blockade" using the complementary HER2 antibodies trastuzumab and pertuzumab results in a significantly higher rate of pathological complete response and disease-free survival (37). The introduction of trastuzumab as an addition to chemotherapy in the treatment of HER2-positive breast cancer significantly improved prognosis in this specific subtype.

1.1.5 Radiotherapy

While breast cancer surgery intends to remove macroscopic disease, the rationale behind radiotherapy (RT) to the breast is that it can destroy residual microscopic (i.e., occult) tumour cells left in the surgical field and thus eliminate undetected multifocal lesions (38). Postoperative RT has shown to decrease local recurrence rates as well as improve patient survival both after BCS (39) and mastectomy in node-positive disease (40). Radiotherapy may be targeting the remaining breast tissue after BCS or the chest wall after mastectomy (local RT), respectively, and regional lymph nodes. Swedish guidelines recommend adjuvant RT to the breast after BCS in most cases and to the chest wall after mastectomy if tumour size exceeds 5 cm or if extensive multifocality was present. Swedish data published in 2018, however, suggest a low-risk BCS group in which postoperative RT to the breast can be safely omitted (41). Regional RT is indicated in the case of node-positive disease (macrometastases) but international guidelines diverge regarding the group of women with 1-3 lymph node metastases and a completion ALND performed. In cases with only micrometastases in the SNB, no regional radiotherapy is indicated and in cases with only one macrometastasis, regional RT can be omitted if the tumour biology is favourable (42). Since RT has known side effects such as increased cardiac mortality and risk of other malignancies such as lung cancer and esophageal cancer (43), predictive biomarkers of response to radiotherapy are being evaluated to avoid overtreatment regarding RT (44,45).

1.2. Breast reconstruction

Mastectomy, especially in younger women, has negative psychological effects with reduced self-esteem, changed body image and sexual problems (46). A reconstruction of the breast, using implants, autologous tissue or a combination of both, can improve body image and quality of life (47). Previously, breast reconstruction was mostly performed as a delayed procedure (DBR) but today, immediate breast reconstruction (IBR) is gaining popularity (48,49). IBR is performed at the same time as the mastectomy, which allows for skin-sparing or nipple-sparing strategies.

1.2.1 Immediate breast reconstruction

In Sweden, an immediate breast reconstruction (IBR) is predominantly implant based. After a mastectomy, the implant can be either placed in a fully subpectoral pocket (often assisted laterally by the serratus fascia) or in the prepectoral space; in addition, a combination can be chosen, replacing muscle coverage at the inferior pole with a mesh or acellular dermal matrix. A permanent implant can be used as a one-stage procedure in women with smaller breast volume, while a tissue expander is commonly used in women with larger breast volume or if skin flaps have appeared compromised during surgery. The use of expander devices often implies a later revision surgery when the expander is changed to a permanent implant (two-stage procedure).

The Swedish National Breast Cancer Register (NKBC) has documented an increase in national IBR rates up to 14% in 2018 (4), which are still low in comparison with many other countries (50–52). There are regional differences with 31% in Stockholm and 11% in the Southern and 9% in the West region which are difficult to explain by geographical conditions, infrastructure and variations in severity of the disease (4).

1.2.2 Delayed breast reconstruction

In DBR, it is possible to use either implants or autologous tissue, or a combination of both. The Latissimus Dorsi (LD) and Deep Inferior Epigastric Perforator (DIEP) flaps are the most common flap-based reconstructions, but fat transplantation is another popular autologous option. The DIEP flap is a free flap from the abdomen transferred to the mastectomy site and was introduced in 1994 (53). Another option is delayed-immediate breast reconstruction which could be used in cases with a risk of radiotherapy after mastectomy. This is a two-stage procedure with first (stage I) a skin-sparing mastectomy with insertion of an implant, and in those patient who did not get radiotherapy (stage II) underwent immediate breast reconstruction and those who get radiotherapy underwent later (stage II) a standard delayed reconstruction (54).

1.3 Predictors affecting the choice of surgical treatment

The choice of surgical intervention in breast cancer is mainly based on patient and tumour characteristics, as well as surgeon skills and patient preferences, even though there are further predictors that may affect surgical strategies.

1.3.1 Tumour and patient characteristics

In preoperative counselling it is important to remember that the full picture of the disease is not yet available, which is especially true for lymph node status in clinical lymph node negativity. The ideal preconditions for BCS would be a relatively small tumour (<3cm), but even large tumours can be safely excised if breast volume and shape allow for oncoplastic volume displacement. Alternatively, volume replacement or partial reconstruction can broaden the indications for BCS, making use of tissues adjacent to the ipsilateral breast moved into the resection cavity as a pedicled flap (perforator flap). For IBR – if a mastectomy is either recommended or desired – several risk factors have to be ruled out, and thorough information should be given on the pros and cons of IBR versus DBR versus no breast reconstruction.

1.3.2 Preoperative information and involvement in the surgical decision-making process

The quality of the preoperative information communicated by the surgeon and the breast care team and the discussion of the different treatment options during the decision-making process preceding the scheduling of a surgical procedure may differ considerably and have a significant impact on patient decisions and postoperative regret (55–57). A higher level of participation in the decision-making process leads to an increased post-decision quality of life and higher satisfaction (58).

1.3.3 Socioeconomic factors

The association between socioeconomic factors and the stage of the breast cancer at diagnosis, as well as the association between socioeconomic factors and surgical treatment choices are well documented, where ethnicity, income and education all play a significant role (59–61). Studies investigating how socioeconomic factors are associated with patient-reported preoperative information regarding surgical alternatives, however, are lacking (62,63), even though low satisfaction with information prior to breast cancer surgery is associated with an increased likelihood of experiencing anxiety and postoperative regret (57,64).

Alderman et al showed that only 33% of women under the age of 80 years undergoing breast cancer surgery had preoperatively discussed breast reconstruction: surgeons were significantly more likely to have this discussion with younger, more educated patients with larger tumours (55). In another U.S. study, received information on IBR prior to surgery was affected by ethnicity and education, but data were based on retrospective chart review (65).

1.4 The effects of radiotherapy on implant-based reconstruction

Radiotherapy (RT) affects the choice and planning of any reconstruction due to its effect on the surrounding tissues such as underlying muscle, skin and subcutaneous fat. RT effects involve chronic inflammatory changes, tissue remodelling and fibrosis (66). One well-known complication after implant-based IBR is capsular contracture, associated with pain, impaired cosmetic outcome and psychological symptoms (67). The risk of surgical complications is 4.2 times higher in patients receiving RT before or after implant-based breast reconstruction in a meta-analysis of 1105 patients (68). In addition, a higher risk for wound complications and infection is seen after later ipsilateral adjustment surgery because of the negative affect of RT on wound healing and tissue repair (66). With about 11-37% reconstruction failure in patients with implants and PMRT, the adverse effect of radiotherapy is well documented (69,70). It has been debated whether IBR is indicated in the face of PMRT, but it is now internationally agreed that PMRT poses no contraindication to IBR in the well-informed patient (71). In a publication from our own group, 77.7% of women receiving PMRT after IBR would still recommend IBR to other women in their situation despite an implant failure rate of 15% (70). In this publication, however, implant failure included both implant removal due to surgical complications and due to the patient's desire to convert an implant-based IBR to an autologous re-reconstruction.

1.4.1 Implant exchange

Even though most tissue expanders can be left in situ for any amount of time without the necessity of an implant exchange, they are commonly replaced with a permanent implant 6-12 months after completion of PMRT. The optimal timing of the expander-implant exchange has been widely discussed (72). Hypothetically, early irradiation effects need to settle before attempting any implant adjustment surgery (73–75). Other factors to take into consideration that may affect the outcome after implant exchange surgery are implant type, complications and lifestyle risk factors. An important clinical question is to what degree the timing of expander-implant exchange in relation to PMRT increases the risk of surgical complications, since unnecessary exposure to significant risks such as implant removal must be carefully considered (74,75).

1.4.2 Capsular contracture

In case of severe capsular contracture, revision surgery commonly involves incision or removal of the capsule (capsulotomy or capsulectomy) with an exchange of the implant or a conversion to a fully or partly autologous re-reconstruction. Potential explanations of the underlying aetiology of radiation-induced fibrosis by e.g. genetic variation are as yet not conclusive (76). Variations in gene expression patterns in irradiated recipient vessels from autologous flap reconstructions (77), as well as in adipose tissue from the irradiated breast (78) have previously been described by our group, but to our knowledge there is no study that has investigated gene expression patterns in irradiated breast capsular tissue. In order to minimize the capsular contracture in the setting of aesthetic breast augmentation, several strategies have been proposed to reduce capsular inflammation (79). Capsular contracture after aesthetic breast augmentation, in the absence of irradiation, is associated with inflammatory cell recruitment together with an increased expression of toll-like receptor 4 (TLR4) (80) and cysteine leukotriene receptor 2 (CysLTR-2) (81) in fibroblasts within the capsular tissue. Further investigation of the basic components in the innate and adaptive immune responses, with focus on specific T and B cells, would be of great interest. Leukotriene inhibitors have been suggested as a prophylactic treatment with the goal of reducing the risk of capsular contracture in aesthetic surgery (82). Furthermore, interleukin 8 (IL8) and metallopeptidase 4 (TIMP4) have been suggested as potential key diagnostic and prognostic biomarkers (83). While most studies have been conducted in purely aesthetic surgery, PMRT after reconstruction can further enhance inflammatory cell recruitment and fibrosis. The Wnt signalling pathway, previously known for pathogenesis of radiation-induced fibro-proliferation as showed by Lipa et al, may play an important part in capsular contracture after breast reconstruction using expander implants followed by PMRT (84). The study, however, was extremely small and did not evaluate gene expression patterns. Due to the incomplete understanding of the pathogenesis of radiation-induced inflammation and the development of capsular contracture in breast cancer patient led us to investigate differences in gene expression patterns and immune cell composition in irradiated versus non-irradiated breast capsular tissue.

2 AIMS OF THE THESIS

The specific aims were:

I. To analyse the causes of regional variations in IBR rates in Sweden through consideration of tumour data together with patient-reported experiences of information and decision-making before surgery,

II. To investigate potential associations between socioeconomic factors and IBR rates, as well as patient-reported preoperative information and involvement in the surgical decision-making process,

III. To explore associations between socioeconomic factors and breast conservation rates, together with patient-reported preoperative information and involvement in the surgical decision-making process,

IV. To assess how the timing of implant revision surgery after the completion of PMRT as well as other clinical risk factors affect surgical complications resulting in implant failure,

V. To identify differences in gene expression patterns and immune cell composition in irradiated versus non-irradiated breast capsular tissue to better understand the underlying biology of radiation-induced capsular contracture.

3 METHODS

3.1 Patients and methods

3.1.1 Study I-III

The first three studies were based on a retrospective cohort design, including the complete annual cohort of women registered for breast surgery due to a primary breast cancer in Sweden in 2013. In study I and II, the subgroup of women with a mastectomy as the final surgical intervention were selected, while women with any type of surgical treatment were included in study III. In bilateral cases, one breast was randomly selected. Data on patient and tumour characteristics as well as received treatment were extracted from the Swedish National Breast Cancer Register, which includes 99–100% of all new breast cancer patients (85,86). No data on DBR was available since there is no national register providing this information.

A postal questionnaire asking for patients' experiences of preoperative information about their surgical and reconstructive options as well as their perceived involvement in the surgical decision-making process were dispatched in spring 2015. The questionnaire also covered hereditary risks and asked for the patients' own view on requesting a breast reconstruction or not. Questions regarding any preoperative discussion of breast-conserving options as an alternative to mastectomy were only used in study III. Postal addresses were retrieved from the tax authority via personal identification numbers. Non-responders were sent a reminder after three months, and data collection was closed on October 30, 2015. Questionnaire data were then linked to individual patients' tumour data. A separate questionnaire was sent to all breast surgeons registered as members of the Swedish Association of Breast Surgery, and included questions on reconstructive skills and experience as well as local availability of reconstructive surgery services. The questionnaire was anonymous and could not be traced to any individuals, hospitals or departments.

In preparation for study II and III, which were conceived based on the results of study I, data from the Swedish National Breast Cancer Registry were updated by a new data extraction in 2016; therefore, cases numbers were completed by late incoming registrations which increased the cohort size analysed in studies II and III. Socioeconomic background data were received from the Swedish Total Population Registry (TPR), the Register on Income and Taxes (IoT), the Longitudinal Integration Database for Health Insurance and Labour Market Studies (LISA), the Swedish Register of Education, and the Swedish Occupational Register, all maintained by the Central Bureau of Statistics Sweden, and linked with NKBC and questionnaire data. Personal data were de-identified before receipt and analysis.

The Ethical Review Authority at Karolinska Institutet approved study I in 2014 (2014/2106-31/1) and study II and III, including amendments in order to update the original database, in 2016 (2016/373-32, 2016/805-32, 2016/2202-32).

3.1.2 Study IV

A complete retrospective cohort of women who had undergone implant exchange and/or capsulotomy or capsulectomy at Karolinska University Hospital between 2005 and 2015 was identified through individual codes for performed surgery registered according to the Classification of Health Interventions. Incorrectly coded or purely prophylactic cases, secondary breast reconstructions and other types of revisions not involving intrusion into the implant cavity were excluded. Thus, remaining patients all had a previous therapeutic mastectomy and an implant-based IBR. Any implant failures occurring before a first surgical revision, i.e. in the postoperative period following the primary surgery, were excluded from analysis; likewise, if implants were removed due to a patient's own preference or in the context of a conversion to an autologous breast re-reconstruction, these cases were also excluded.

Information on tumour characteristics, surgical and oncological treatment, lifestyle risk factors and comorbidity (smoking, BMI, hypertension, immunosuppressive medications) were collected through individual medical chart review and entered into the electronical Stockholm Breast Reconstructive Database. The main outcome was implant failure due to postoperative complications associated with revision surgery after IBR. Time was calculated from the last day of RT to the date of revision surgery. In case of multiple revision surgeries, each intervention counted as one separate case, with the time corresponding to each individual date of revision surgery.

The original study was approved by the Ethical Review Authority at Karolinska Institutet, Stockholm, in 2015 (2015/1183-31/4).

3.1.3 Study V

3.1.3.1 Human tissue samples

A total of 44 patients (46 breasts), who underwent an implant exchange or a conversion to an autologous reconstruction after an IBR, were included after signing an informed consent. Irradiated (n=23) and non-irradiated (n=23) 10 mm x 10 mm biopsies were collected from the lateral lower quadrant capsule at two institutions in Stockholm. Each biopsy was divided into to two parts, one stored in formalin for immunohistochemistry and Allprotect Tissue Reagent® (Qiagen, Hilden, Germany) and kept at –80 °C for RNA purification.

The Ethical Review Authority at Karolinska Institutet approved this study in 2017 (2017/1504-31/2) and all samples were registered and stored in the Stockholm Medical Biobank (nr 914).

3.1.3.2 Extraction of RNA

Extraction of RNA was performed using The RNeasy Lipid Tissue kit ® (Qiagen) according to the protocol of the manufacturer. Microcapillary electrophoresis was performed to evaluate RNA quality, by using an Agilent Bioanalyzer® with RNA 6000 Pico Kit and Agilent 2200 TapeStation with RNA ScreenTape (Agilent, Santa Clara, California, USA). While ultraviolet spectrophotometry was performed to measure the RNA quantity, by using NanoDrop® ND-1000 UV-Vis Spectrophotometer (Thermo Scientific, Waltham, Massachusetts, USA). The whole-transcriptome expression was retrieved by processing 50ng of each RNA sample, using GeneChip® WT Pico Reagent Kit (Affymetrix, Santa Clara, California, USA), which produces amplified and biotinylated sense-strand DNA targets for hybridization to Clariom D arrays.

3.1.3.3 Profiling of gene expression

The gene expression was assessed by microarrays analysis, Affymetrix® Clariom D human oligonucleotide microarrays (Affymetrix, Santa Clara, California, USA), and carried out by the core facility for Bioinformatics and Expression Analysis at Karolinska Institutet. The scanning from the files were processed in Transcriptome Analysis Console using the Signal Space Transformation-Robust Multi-Chip Analysis method. The transcripts registered as encoded genes according to The National Center for Biotechnology Information (NCBI)'s database for gene-specific information, Entrez Gene, http://www.ncbi.nlm.nih.gov/gene , were extracted if present in at least one of the irradiate or non-irradiated groups. The most differentially expressed genes between the irradiated and the non-irradiated samples were identified.

Enrichment testing was performed in order to identify different levels of gene sets and pathways associated to RT-responsive genes, by using The Molecular Signatures Database (MSigDB, https://www.gsea-msigdb.org/gsea/msigdb/index.jsp).

3.1.3.4 Evaluation of immune cells

To assess the relative levels of specific immune cell types within the complex gene expression mixture, based on the RNA expression patterns, the analytical platform CIBERSORT (https://cibersort.stanford.edu/) was used. Immune cell type differences between the irradiated and the non-irradiate groups were also compared.

3.1.3.5 *Immunohistochemistry*

The formalin-fixed tissue samples were embedded in paraffin after being processed in an automated tissue processing machine (VIP 3000, Miles Scientific). The 4-µm-thick sections were then mounted on glass slides (Superfrost+, Thermo Scientific) and heated for 3 hours at 56°C. Each section de-paraffinized in xylene and rehydrated in alcohol before a heat-induced epitope retrieval was performed using a Decloaking Chamber (Biocare Medical) set for 5 min at 110°C in Citrate buffer pH 6 (Sigma C-9999). To stop the endogenous peroxidase, a 30-minute incubation in 0.15 % hydrogen peroxidase was performed at room temperature, followed by a 30-minute blocking step using 1% bovine serum albumin (BSA). Primary antibodies were diluted 1/2000 (CD3), 1/600 (CD20), 1/1000 (CD68), in 1% BSA and incubacted at +4°C overnight in a humid chamber. The secondary biotinylated antibody (Vector Laboratories) was diluted 1/200 and incubated for 30 min at room temperature, followed by 30 min incubation with avidin-biotin enzyme complex (Vectastain) ELITE ABC kit (HRP, Vector Laboratories). The peroxidase substrate DAB (ImmPACT DAB SK-4105, Vector Laboratories) was used for 3 minutes for visualization. Each section was then counterstained in Mayer's hematoxylin for 1 min followed by dehydration with graded alcohols, xylene and cover slipped with Mountex. Alongside the capsular surface, three high power fields (x20) were chosen with a depth of 200 µm below the surface. Staining of capsular biopsies for CD3+, CD20+ and CD68+ were performed. CD3+ is a T cell co-receptor and CD68+ is highly expressed in macrophages. CD20+ is expressed on the surface of B cells, which is a essential for the adaptive part of the immune response. Two blinded evaluators independently counted the numbers of CD3-, CD20- and CD68-positive cells.

3.2 Statistical analysis

As an overall standard, the distribution of data was tested for normality before further analysis, and parametric or non-parametric tests used accordingly. In study I-IV, categorical data were presented as case numbers per group with their percentages, and groups compared by the Chi Square or Fisher's exact test. When presenting continuous variables, median values and their range were used. For comparisons between groups, the Mann-Whitney (two groups) or the Kruskal-Wallis test (more than two groups) was employed.

Uni- and multivariable logistic regression analyses were performed when testing associations of potential factors with a binary outcome. Results are presented as odds ratios (OR) with their respective 95% confidence intervals (Cl). SPSS® version 24 (IBM, Armonk, New York, USA) was used to perform all analyses with statistical significance set at the 0.05 level.

3.2.1 Study I-III

In study I and II, the original mastectomy cohort was divided into two groups: women with and those without an IBR. The NKBC cT status "cT0" ("no obvious tumour") was categorized as cT1 since cT0 is normally based on palpation only instead of radiological findings, as opposed to cTis (in situ only). In order to identify patients with a low likelihood of PMRT according to preoperative parameters, clinically node-negative patients aged 65 years or less with small tumours (cT1), in situ disease only (cTis) or no signs of a primary tumour (cT0) were selectively defined as "low tumour burden" group. A separate analysis was also performed to compare responders with non-responders (study II).

In study III, the original cohort included all types of breast surgery, and two groups were created for comparison: patients treated with breast-conserving surgery and those treated with mastectomy (with or without IBR). Since IBR patients were found to differ from mastectomy patients without IBR in several aspects, analysis was first conducted on all mastectomy patients, i.e. including those with an IBR, and then completed with a second analysis excluding IBR patients. For the specific purposes of study III, the following questions were selected from the patient questionnaire: "Did your surgeon discuss the option of breast-conserving surgery?" (Yes/Yes, partly/No), "Who took the decision to choose mastectomy?" (My choice, Surgeon's choice, Both), and "Did you feel involved in the decision-making process to choose mastectomy?" (Yes/Yes, partly/No). The alternatives "Yes" and "Yes, partly" as well as "My choice" and "Both" were then merged for the statistical analysis. In a separate analysis, questionnaire data from patients with invasive tumours were compared between two groups: patients with smaller, clinically node-negative tumours (cT1), likely to be technically suitable for BCT, and patients with clinically larger tumours (cT2-cT4).

Univariable binary logistic regression analysis was performed to study the association of tumour/patient characteristics and information/involvement with performance of IBR versus no IBR in study I. In study II, the binary outcome was the same as well as all included variables, but socioeconomic factors were added to the analysis. For study III, the binary outcome was the performance of BCS versus mastectomy (with or without IBR). Questionnaire data on information/involvement could not be included in the regression analysis in study III, since women with BCS had not participated in the survey. In a subsequent step, all factors selected for univariable analysis were also entered into a multivariable model.

3.2.2 Study IV

After descriptive analysis, data were entered into a univariable binary logistic regression with implant failure versus no implant failure as the endpoint. Performing a multivariable regression was not deemed appropriate due to the limited number of events. In order to reflect the situation in the clinical reality and thus the need of data facilitating preoperative counselling, the implant failure rate was calculated per revision surgery and not per breast or individual.

3.2.3 Study V

The differences in expression levels in irradiated and non-irradiated samples were calculated in groups using moderated t-tests as implemented in the BioConductor Limma kit. Only genes expressed in either of the treatment groups by using Transcriptome Analysis Console while also containing protein-coding exons and being listed in Extrex Gene were included for further analysis. The selected genes for enrichment testing had a p-value <0.05. The Reactome and ontology biological gene sets were considered significantly overrepresented with a corrected a P-value<0.05 and False Discovery Rate <5%.

4 RESULTS

4.1 Study I-III

In study I, 2929 women who had received a mastectomy as the final surgical intervention in 2013 were identified through the NKBC (Table 1 in study I). In 412 women (13.8%), breast conservation was primarily attempted and the mastectomy performed at a second session.

In study II-III, data on 7735 women were received from NKBC, of whom 4604 (59.5%) were operated with BCS and 3131 (40.5%) with mastectomy (Table 1 in study III). Of the latter group, 267 women (8.5%) had received an IBR (Table 2 in study II). For 78 women with bilateral disease, one side was selected at random.

4.1.1 Regional differences in treatment, tumour and patient characteristics (Study I-III)

Pre- and postoperative patient and tumour characteristics by healthcare regions are presented in Table 1 in study I for the mastectomy cohort (with or without IBR) and in Table 3 in study III for all women who underwent breast cancer surgery. The IBR rate was highest in the Stockholm/Gotland region with 25.6% and lowest in the West region with 3.0% (study I) Likewise, BCS rates varied from 66.1% in Stockholm/Gotland to 50.7% in the Southeast region (study III). Even though tumour characteristics differed between regions, no explanatory patterns for BCS or IBR variations could be discerned.

Looking for factors that could explain the regional differences in IBR rates, the association with planned postoperative radiotherapy and age at surgery were specifically analysed (study I). There were significant differences in preoperative tumour stage, rates of neoadjuvant treatment and the frequency of planned postoperative radiotherapy (as discussed at the postoperative multidisciplinary team meeting) in different regions, but these differences could not explain variations in IBR rates. The Stockholm/Gotland region with the highest IBR rate also had the highest rate of neoadjuvant therapy and highest percentage of more advanced tumours (cT3-4), whereas the region with the lowest IBR rate (West) had the highest percentage of smaller tumours and the lowest rate of both neoadjuvant therapy and postoperative radiotherapy. Median patient age did not differ between regions.

Of all women without a reconstruction, 657 out of 1947 (33.7%) reported that they themselves did not wish a breast reconstruction, and 155 (8.0%) wanted to wait with breast reconstruction until later. Since a common argument against offering IBR in several Swedish regions is planned RT, a previously described “low tumour burden” group (see Methods) was selected, consisting only of women with a low likelihood of adjuvant RT after IBR. Even in this subgroup, patient participation in treatment decision and perceived preoperative information still varied extensively between Swedish healthcare regions, contradicting the above assumption that younger women with no risk factors for receiving postoperative RT would be sufficiently informed about IBR, see **Table 1** below (corresponding to Table 4 in Study I). Patient information about delayed breast reconstruction (DBR) was generally higher, even though it also varied significantly in the low tumour burden group.

Table 1. Preoperative patient-reported perception of information about breast reconstruction and involvement in treatment decision, in each Swedish healthcare region.

	Swedish healthcare region						
	North	Stockholm/ Gotland	South	South-east	Uppsala/ Örebro	West	P*
Any age							
Did you receive information about the possibility of immediate reconstruction of the breast?							
Group 1	36 (45·6)	123 (67·2)	120 (48·4)	64 (41·0)	69 (35·8)	63 (31·0)	<0·001
Group 2	16 (25·0)	130 (56·3)	60 (27·8)	43 (33·6)	68 (26·4)	53 (24·8)	<0·001
Did you receive information about the possibility of delayed reconstruction of the breast in a second setting?							
Group 1	47 (59·5)	127 (67·9)	160 (65·3)	91 (57·6)	122 (62·6)	127 (62·6)	0·008
Group 2	33 (49·3)	157 (67·7)	120 (55·3)	74 (57·4)	135 (52·5)	130 (60·7)	0·425
Did you feel involved in the decision-making process whether or not to perform breast reconstruction?							
Group 1	52 (72·2)	136 (75·1)	156 (66·1)	88 (59·9)	104 (58·1)	114 (61·3)	0·005
Group 2	33 (53·2)	164 (74·5)	101 (50·0)	61 (54·5)	133 (59·1)	108 (55·1)	<0·001
Age up to 65 years							
Did you receive information about the possibility of immediate reconstruction of the breast?							
Group 1	28 (58·3)	95 (83·3)	84 (59·6)	48 (56·5)	55 (47·8)	35 (34·3)	<0·001
Group 2	12 (36·4)	98 (73·7)	40 (35·7)	34 (42·5)	45 (36·3)	29 (27·1)	<0·001
Did you receive information about the possibility of delayed reconstruction of the breast in a second setting?							
Group 1	40 (83·3)	97 (84·3)	118 (84·9)	70 (81·4)	102 (87·2)	85 (82·5)	0·903
Group 2	26 (74·3)	119 (88·8)	91 (81·3)	66 (81·5)	99 (80·5)	88 (81·5)	0·314
Did you feel involved in the decision-making process whether or not to perform breast reconstruction?							
Group 1	36 (76·6)	94 (83·9)	100 (72·5)	57 (68·7)	78 (68·4)	57 (62·6)	<0·001
Group 2	17 (53·1)	112 (85·5)	58 (53·2)	46 (62·2)	81 (69·2)	62 (61·4)	0·018

Values are numbers of women who answered 'Yes' or 'Yes, but not enough' to each question, with percentages in parentheses. Group 1 (low tumour burden) consisted of women with clinically node-negative cT1, cTis (*in situ*) or cT0 tumours. Group 2 (higher tumour burden) consisted of all remaining women. *χ^2 test.

4.1.2 Socioeconomic factors (Study II and III)

When comparing women who had an IBR with those who had a mastectomy only within the mastectomy cohort (studies I and II), the first were more likely to be in a partnership or married (60.7%), had most often a Swedish background (82.8%) and the highest level of education (35.6 %), and were most often employed as clerks or civil servants (54.7%) with a high income per household (55.1%; Table 3 in study II). In table 4 in study II, the six Swedish healthcare regions are compared: socioeconomic background, tumour data and IBR rates differed significantly among women treated with mastectomy. The region of Stockholm/Gotland, with the highest IBR rate of 25.6%, had the lowest rate of small tumours and the youngest age at surgery, but also the highest rate of non-Swedish born women, a high level of education, fewest unemployed or retired individuals, and the largest high-income group.

When extending the cohort to all types of breast surgery (study III), women receiving BCS were more often in a partnership or married (58.1%) and had a middle or high income (35.3% and 35.4%, respectively) than patients with a mastectomy with or without IBR. In table 3 in study III, the regional distributions of all variables and the significant variation of BCS rates are reflected. Even though tumour characteristics differed between regions, no explanatory patterns for BCS variations could be discerned. As illustrated, Stockholm/Gotland clearly differed from the other regions in all socioeconomic factors. Since only those women operated by mastectomy, not those treated with BCS, had been sent a questionnaire regarding perceived patient information and involvement in the treatment decision, questionnaire results were selectively analysed. Women who felt that the mastectomy decision was their own choice were older ($p<0.001$), had smaller tumours (cT1, $p<0.001$) and fewer axillary metastases ($p<0.001$), lived more often in the North region ($p=0.033$) and were less often registered as working as labourers ($p < 0.001$). Women who did not feel the choice was theirs but still felt involved in the decision were significantly older than those who did not ($p=0.034$), and had smaller tumours without axillary metastases ($p=0.001$). Women who reported that BCS had been discussed as an alternative to mastectomy did not differ in age or region of residence, but had smaller tumours without axillary metastases ($p<0.001$), lived more often in a partnership ($p<0.001$), had an employment ($p=0.031$) and were more often not born in Sweden ($p=0.035$). They also had a tendency to have a higher income but this did not reach statistical significance ($p=0.051$). When selectively analysing women with clinically smaller tumours (cT1) who should have been technically feasible candidates for BCS, rates of preoperative information on BCS and perceived involvement were surprisingly low and varied significantly in different health care regions, see **Table 2** below (corresponding to Table 5 in study III). Overall, lower socioeconomic status was associated with larger clinical tumour size ($p<0.001$ for all variables) as was being born outside Europe (median invasive tumour size 19 mm versus 16 mm, $p=0.002$). In the latter subgroup, axillary lymph nodes were significantly more often clinically positive than in women born in Sweden and Europe (16% versus 9.8% and 11.8%, respectively; $p < 0.001$). Of women born in Sweden and women born in Europe, 14.1% and 12.7% perceived the decision of mastectomy as their own or theirs together with the surgeon, while the corresponding figure was only 9% for women born outside Europe ($p=0.002$). Excluding IBR patients from the mastectomy group, no difference in perceived involvement in the mastectomy decision was seen between different regions of origin ($p=0.162$), while all other factors diverged even more strongly.

Table 2. Patient-reported preoperative information about breast-conserving surgery and perceived involvement in the surgical decision among women treated with mastectomy (with or without IBR) in each Swedish healthcare region.

All ages	North	Stockholm/Gotland	South	Southeast	Uppsala/Örebro	West	*p*
Did your surgeon discuss the option of breast-conserving surgery?[a]							
cT1	60 (80.0)	83 (53.5)	115 (54.8)	95 (58.6)	100 (55.6)	121 (61.4)	0.002
cT2–4	23 (39.7)	90 (43.5)	78 (42.9)	36 (35.0)	88 (39.8)	70 (38.7)	0.730
Who took the decision to choose mastectomy?[b]							
cT1	56 (76.7)	94 (60.3)	138 (63.9)	110 (66.3)	109 (58.9)	130 (64.0)	0.132
cT2–4	37 (62.7)	124 (59.3)	91 (47.9)	58 (53.7)	122 (52.8)	95 (51.6)	0.182
Did you feel involved in the decision-making process to choose mastectomy?[a]							
cT1	66 (90.4)	123 (78.8)	179 (81.7)	149 (90.0)	163 (87.6)	168 (82.4)	0.014
cT2–4	49 (81.7)	175 (82.9)	145 (76.7)	87 (82.1)	186 (80.2)	150 (80.6)	0.737

[a]Values are the number of women who answered "Yes" or "Yes, partly" to each question, with percentages in parentheses

[b]Values are the number of women who answered "My choice" and "Both", i.e. patient's and surgeon's choice, with percentages in parentheses. Patients with in situ disease only were excluded

4.1.3 Socioeconomic differences in questionnaire responders versus non-responders

The response rate to the postal questionnaire was 76.3% (2217 of 2906) after one postal reminder. Of 2217 responders, data for 46 individuals were lost on linkage to socioeconomic data. In study II, a comparison between 2171 responders and 960 non-responders showed that the first group comprised younger women with more favourable tumour disease, a slightly lower rate of IBR and a higher socioeconomic status (Table 1 in study II).

4.1.4 Education and skill level in reconstructive techniques among breast surgeons and availability of in-house plastic surgery services

Ninty-one out of 151 breast surgeon (60.3%) registered as members of the Swedish Association of Breast Surgery answered the anonymous questionnaire about skill level in reconstructive techniques. Five surgeons reported they had either retired or stopped working with breast cancer patients, resulting in 86 completed questionnaires. The majority of responders were senior surgeons with more than five years as a consultant (72 of 86; 84%), and with breast surgery representing more than 50% of their daily clinical activities (64 of 86; 74%). Eight (9%) reported being trained in plastic surgery. Forty-two% (36 out of 86) of the responders could perform an IBR independently, and 76% reported IBR availability at their own hospital. A large majority (81%) wished to receive more training in oncoplastic and reconstructive breast surgery. Median age was 55 (range 34-71) years, and the distribution between regional, county and university hospitals was even.

The availability of in-house plastic surgery services was reported per hospital where patients had been operated. Availability varied between 95.3% (calculated per patient) in Stockholm/Gotland and 30.0-57.5 % in other regions. While this did not suffice to explain regional differences, it increased the likelihood of being informed about IBR options (HR 0.74, 95% CI 0.62 to 0.87). The rate of IBR was higher in regional hospitals without a department of plastic surgery but with a plastic surgeon available than in university hospitals with an own department of plastic surgery (17.2 versus 11.5%; $p<0.001$). Without any plastic surgery service, the IBR rate fell to 3.0%.

4.1.5 Independent predictors of receiving BCS or IBR

Independent predictors for undergoing BCS were a smaller tumour, clinically uninvolved lymph nodes, living in the North or the Stockholm/Gotland region, a higher education and a higher income, see **Table 3** below (corresponding to Table 4 in study III). When running multivariable regression analysis excluding those women having received an IBR, the oldest together with the youngest age group showed the lowest probability to receive BCS, and having the highest level of education did no longer act as an independent predictor of BCS (OR 1.18, 95% CI 0.98–1.41). Living in the Stockholm/Gotland region resulted in a significantly increased likelihood of BCS compared to the reference region North (OR 1.39, 95% CI 1.10–1.76).

Younger age, non-invasive disease, no clinically involved lymph nodes and residing in the Stockholm/Gotland region were independent predictors of undergoing IBR, as well as the availability of in-house plastic surgery services, patient information and involvement in decision-making. An important question was if the observed regional differences were associated with differences in socioeconomic background. To assess this, uni- and multivariable analyses were performed. Socioeconomic factors that independently increased the likelihood of having an IBR were living in a single household, being employed, and having a higher income, see **Table 4** below (corresponding to Table 5 in study II). To be single appeared to decrease the likelihood of IBR in the univariable analyses, but this association reversed when adjusted for age, as being single strongly interacted with younger age. Despite these adjustments for socioeconomic status, the single most important independent predictor remained patient-reported preoperative information about the possibility of IBR. Patient-reported involvement in the surgical decision-making process was also confirmed as a significant independent factor for IBR.

Table 3. Univariable and multivariable binary logistic regression analysis of clinical and socioeconomic factors with performance of breast-conserving surgery as opposed to mastectomy (with or without IBR) as the binary endpoint.

	Univariable		Multivariable	
	Odds ratio	*p*	Odds ratio	*p*
Age (years)				
≤40	1.00 (reference)			
41–50	1.74 (1.36–2.21)	<0.001	1.58 (1.19–2.10)	0.001
51–65	2.75 (2.19–3.45)	<0.001	2.36 (1.80–3.09)	<0.001
>65	1.62 (1.29-2.02)	<0.001	1.74 (1.30–2.33)	<0.001
Preoperative clinical tumour stage				
cTis (in situ only)	1.00 (reference)			
cT1 (≤20 mm)	1.66 (1.33–2.07)	<0.001	1.87 (1.49–2.35)	<0.001
cT2 (21–50 mm)	0.33 (0.26–0.42)	<0.001	0.42 (0.33–0.54)	<0.001
cT3 (>50 mm)	0.05 (0.03–0.08)	<0.001	0.07 (0.04–0.11)	<0.001
cT4	0.05 (0.02–0.13)	<0.001	0.10 (0.04–0.27)	<0.001
Preoperative node status				
cN0	1.00 (reference)			
cN1	0.23 (0.19–0.27)	<0.001	0.40 (0.33–0.48)	<0.001
Family status				
Partnership/married	1.00 (reference)			
Single	0.81 (0.74–0.89)	<0.001	1.01 (0.87–1.17)	0.901
Own birth country				
Sweden	1.00 (reference)			
Europe, not Sweden	1.17 (1.00–1.37)	0.054	1.38 (1.14–1.67)	0.001
Outside of Europe	0.85 (0.68–1.06)	0.142	1.23 (0.93–1.61)	0.144
Highest level of education				
Primary school	1.00 (reference)			
Secondary school	1.64 (1.46–1.85)	<0.001	1.33 (1.16–1.53)	<0.001
Post-secondary school education, 3 years or less	1.67 (1.43–1.96)	<0.001	1.31 (1.10–1.59)	0.004
Post-secondary school education, more than 3 years	1.54 (1.35–1.77)	<0.001	1.20 (1.00–1.42)	0.044
Occupation				
Clerk/civil servant	1.00 (reference)			
Entrepreneur	1.02 (0.78–1.33)	0.891	1.03 (0.76–1.40)	0.831
Labourer	1.04 (0.90–1.21)	0.581	1.11 (0.93–1.34)	0.256
Unemployed/retired	0.75 (0.67–0.83)	<0.001	0.95 (0.79–1.13)	0.539
Income per household				
Low	1.00 (reference)			
Middle	1.60 (1.43–1.78)	<0.001	1.39 (1.19–1.62)	<0.001
High	1.57 (1.40–1.75)	<0.001	1.29 (1.05–1.58)	0.014
Region				
North	1.00 (reference)			
Stockholm/Gotland	1.07 (0.89–1.30)	0.473	1.05 (0.84–1.31)	0.660
South	0.72 (0.60–0.87)	0.001	0.67 (0.54–0.83)	<0.001
Southeast	0.57 (0.46–0.70)	<0.001	0.48 (0.38–0.62)	<0.001
Uppsala/Örebro	0.79 (0.66–0.96)	0.015	0.82 (0.66–1.02)	0.070
West	0.75 (0.62–0.91)	0.004	0.71 (0.57–0.88)	0.002

Values in parenthesis are 95% confidence intervals

Table 4. Univariable and multivariable binary logistic regression analysis of clinical and socioeconomic factors, with performance of immediate breast reconstruction as opposed to mastectomy without reconstruction as the binary endpoint (mastectomy patients only).

	Univariable analysis		Multivariable analysis	
	Hazard ratio	*P*	Hazard ratio	*P*
Age (years)				
≤ 40	1·00 (reference)			
41–50	0·85 (0·58, 1·26)	0·425	0·88 (0·43, 1·78)	0·715
51–65	0·38 (0·25, 0·56)	< 0·001	0·54 (0·27, 1·09)	0·087
> 65	0·04 (0·02, 0·06)	< 0·001	0·11 (0·04, 0·30)	< 0·001
Preoperative cT category				
cTis (*in situ* only)	1·00 (reference)			
cT1 (≤ 20 mm)	0·21 (0·14, 0·30)	< 0·001	0·42 (0·23, 0·77)	0·005
cT2 (21–50 mm)	0·12 (0·08, 0·18)	< 0·001	0·23 (0·12, 0·45)	< 0·001
cT3 (> 50 mm)	0·15 (0·09, 0·27)	< 0·001	0·31 (0·13, 0·74)	0·008
cT4	0·18 (0·07, 0·49)	0·001	0·29 (0·02, 3·49)	0·326
Preoperative cN category				
cN0	1·00 (reference)			
cN1	0·34 (0·22, 0·55)	< 0·001	0·30 (0·14, 0·67)	0·003
Family status				
Partnership/married	1·00 (reference)			
Single	0·70 (0·54, 0·91)	0·007	1·81 (1·04, 3·17)	0·037
Own birth country				
Sweden	1·00 (reference)			
Europe, not Sweden	0·90 (0·56, 1·46)	0·677	0·97 (0·44, 2·14)	0·940
Outside Europe	2·29 (1·47, 3·56)	< 0·001	0·83 (0·37, 1·87)	0·653
Highest level of education				
Primary school	1·00 (reference)			
Secondary school	2·64 (1·74, 4·00)	< 0·001	0·62 (0·32, 1·20)	0·155
Postsecondary school, ≤ 3 years	2·94 (1·79, 4·83)	< 0·001	0·76 (0·35, 1·66)	0·498
Postsecondary school, > 3 years	5·03 (3·29, 7·68)	< 0·001	0·85 (0·41, 1·75)	0·659
Occupation				
Clerk/civil servant	1·00 (reference)			
Entrepreneur	0·46 (0·23, 0·90)	0·024	0·63 (0·22, 1·85)	0·403
Labourer	0·68 (0·49, 0·95)	0·022	0·94 (0·55, 1·63)	0·830
Unemployed/retired	0·10 (0·07, 0·15)	< 0·001	0·52 (0·27, 1·00)	0·049
Income per household				
Low	1·00 (reference)			
Middle	2·47 (1·69, 3·63)	< 0·001	1·90 (0·97, 3·70)	0·061
High	5·02 (3·53, 7·14)	< 0·001	2·79 (1·25, 6·22)	0·012
Region				
North	1·00 (reference)			
Stockholm/Gotland	7·59 (3·92, 14·69)	< 0·001	6·62 (2·70, 16·20)	< 0·001
South	1·09 (0·52, 2·25)	0·825	0·98 (0·38, 2·51)	0·958
South-East	1·41 (0·66, 3·02)	0·374	1·45 (0·54, 3·94)	0·462
Uppsala/Örebro	1·42 (0·70, 2·90)	0·329	1·32 (0·50, 3·44)	0·577
West	0·52 (0·23, 1·18)	0·119	0·57 (0·19, 1·77)	0·334
Received preoperative information				
Yes	1·00 (reference)			
No	32·99 (18·26, 59·59)	< 0·001	12·73 (6·03, 26·89)	< 0·001
Involved in decision-making				
Yes	1·00 (reference)			
No	13·71 (7·21, 26·07)	< 0·001	2·56 (1·14, 5·76)	0·023

Values in parentheses are 95 per cent confidence intervals.

Independent predictive factors for reporting having received preoperative information about IBR were having a non-invasive tumour (OR 3.56, 95% CI 2.29-5.55), living in the Stockholm/Gotland region (OR 2.64, 95% Cl 1.70-4.11) and being born outside Europe (OR 2.83, 95% Cl 1.68-4.77). Negative predictive factors were being more than 65 years old (OR 0.43, 95% Cl 0.26-0.71) and having no current employment (including retirement) (OR 0.69, 95% Cl 0.49-0.97). The small group of 154 women with a non-European background, mostly born in Asia, were younger and had a higher educational level than Swedish or European-born women, and most of them lived in the Stockholm/Gotland area.

4.2 Study IV

The final cohort consisted of 475 breast cancer patients (542 breasts) in whom 707 implant revision surgeries had been performed (Figure 1 in study IV). Median follow-up time, i.e. time from revision surgery to medical chart review, was 95 months (range 2-215). For details on patient and tumour characteristics per breast, see Table 1 in study IV.

Due to a previous breast cancer treated by breast conservation (N=23) or due to lymphoma (N=1), twenty-four breasts (4.4%) had been irradiated between 1 and 25 years prior to IBR. In a further 223 cases (41.1%), the chest wall had been irradiated after mastectomy (PMRT) and IBR, while 288 breasts (53.1%) had never been irradiated. In seven cases and eight revision surgeries, information on irradiation was missing and these cases were therefore excluded. Due to surgical complications, a total of 33 implants (one bilateral case of implant failure) were removed, see **Figure 1** (corresponding to Figure 1 in study IV). In the group of non-irradiated breasts, the implant failure rate was lowest with 2.4% (9 out of 375). Corresponding figures were 7.5% (22 out of 293) in the PMRT group and 6.5% (2 out of 31; overall p=0.008) in the group with RT given prior to IBR. In most cases (76.8%), only one revision surgery had been performed, with a median of one revision surgery involving the implant cavity (range 1-5) during follow-up. The median time from revision to implant failure was two months (1-153).

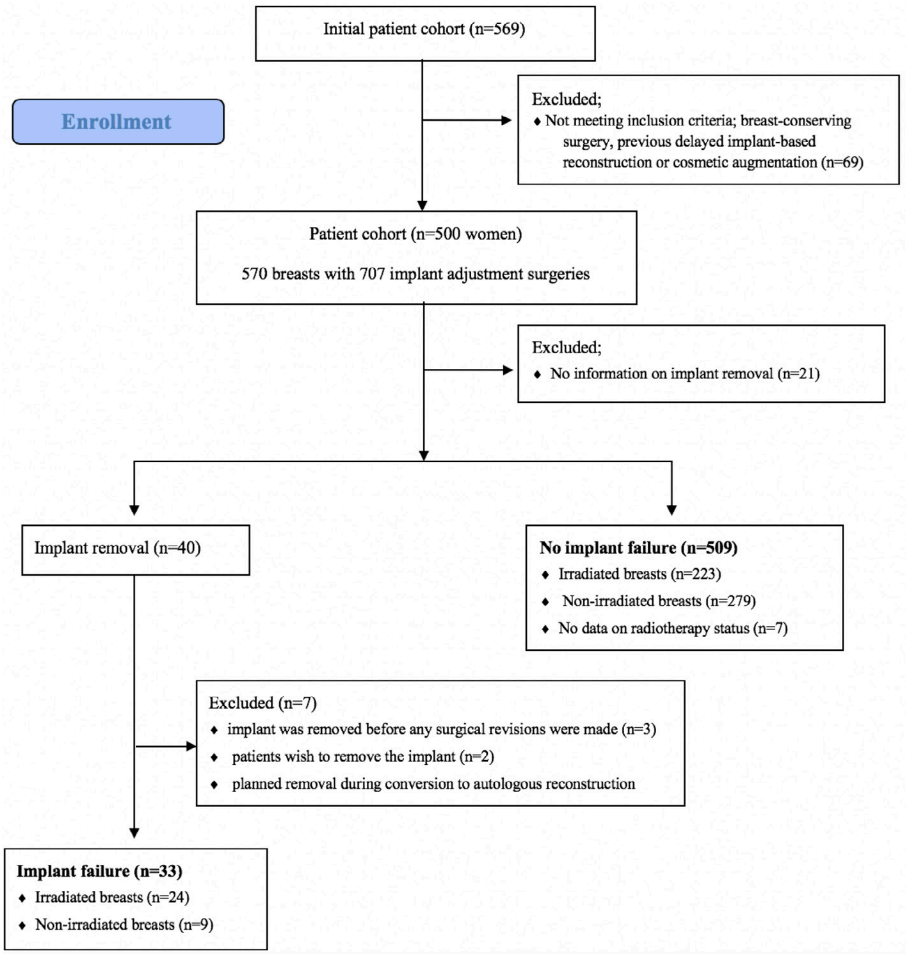


Figure 1. *Flow chart for the creation of the final cohort of cases of implant removal (N=33) and no implant (N=509)*

Median time from completion of any RT (PMRT or prior to IBR) to revision surgery was 14.5 months (1-338); the cohort was accordingly divided into two groups using the median as a cut-off. Among cases of implant failure, 42.1% had revision surgery performed less than 14.5 months after RT completion, and 57.9% after more than 14.5 months (p=0.633). Thus, no significant impact of timing of revision surgery could be identified. Please see Table 2 in study IV for radiotherapy details per breast.

In **Table 5** (corresponding to Table 3 in study IV), unadjusted risk factors for implant failure, calculated per breast, are presented. Known risk factors such as current smoking and antidiabetic treatment were confirmed in the present cohort.

A significantly increased risk of implant failure after revision surgery was seen in patients with an infection after the initial operation, namely mastectomy and IBR. The increased risk was seen regardless of whether the previous infection was clinically diagnosed or confirmed by bacterial cultures and/or elevated inflammatory markers such as the C-reactive protein. This is noteworthy since these infections had obviously not resulted in an implant failure during the postoperative period following IBR.

Table 5. Univariable logistic regression analysis with breast implant failure as the endpoint.

	All cases	Implant removal	**Univariable** *Odds ratio*	*P*
Total number of breasts	542	33		
Age (years) at IBR				
< 50	319	21	1.00 (reference)	
50-60	163	10	0.93 (0.43 – 2.02)	0.850
>60	60	2	0.49 (0.11 – 2.14)	0.343
Missing	*0*	*0*		
Histopathological tumour stage				
Tis (in situ only)	135	11	1.00 (reference)	
T1 (≤ 20mm)	55	1	0.21 (0.03 – 1.66)	0.138
T2 (21–50mm)	112	4	0.42 (0.13 – 1.35)	0.144
T3/T4 (> 50 mm)	86	5	0.70 (0.23 – 2.08)	0.516
Missing	*175*	*12*		
Histopathological nodal stage				
Node negative	407	17	1.00 (reference)	
Node positive	111	14	3.31 (1.58 – 6.95)	0.002
Missing	*44*	*2*		
Neoadjuvant chemotherapy				
No	447	26	1.00 (reference)	
Yes	85	7	1.45 (0.61 – 3.47)	0.399
Missing	*10*	*0*		
Axillary lymph node dissection				
No	290	7	1.00 (reference)	
Yes	240	26	4.91 (2.09 – 11.53)	<0.001
Missing	*45*	*0*		
Type of implant				
Temporary expander	33	1	1.00 (reference)	
Permanent expander	434	29	2.29 (0.30 – 17.37)	0.422
Fixed-volume implant	68	2	0.97 (0.09 – 11.10)	0.980
Missing	7	1		
Final implant volume*				
<300 cc	151	8	1.00 (reference)	
300 – 400 cc	265	12	0.85 (0.34 – 2.12)	0.724
>400 cc	118	10	1.66 (0.63 – 4.33)	0.305
Missing	*8*	*3*		
Radiotherapy				
None	288	9	1.00 (reference)	
Yes, prior to IBR	24	2	2.82 (0.57 – 13.85)	0.202
Yes, after IBR	223	22	3.39 (1.53 – 7.53)	0.003
Missing	*7*	*0*		
Type of adjuvant radiotherapy**				
Local	84	3	1.00 (reference)	
Locoregional	141	19	4.10 (1.18 – 14.32)	0.027
Missing	*7*	*0*		
Radiotherapy dose/fractions**				
46 Gray / 23 fractions	18	2	1.00 (reference)	
50 Gray / 25 fractions	198	20	0.90 (0.19 – 4.20)	0.892
Missing/other	*14*	*0*		
Months from end of radiotherapy to first surgical revision				
≤ 14.5 months	113	8	1.00 (reference)	
> 14.5 months	114	11	1.40 (0.54 – 3.63)	0.486
Missing	*0*	*0*		
Postoperative infection within 30 days after IBR				
No infection	398	10	1.00 (reference)	
Clinical signs of infection, oral antibiotic treatment	72	7	4.18 (1.54 – 11.37)	0.005
Confirmed infection, oral antibiotic treatment^	14	4	15.52 (4.15 – 58.01)	<0.001

Confirmed infection·, intravenous antibiotic treatment ^	12	3	12.93 (3.04 – 55.12)	0.001
Missing	*46*	*9*		
Postoperative complication with return to theatre (within 30 days) after IBR				
No	489	24	1.00 (reference)	
Yes	11	1	1.94 (0.24 – 15.76)	0.536
Missing	*42*	*8*		
Postoperative complication without return to theatre after IBR				
None	464	23	1.00 (reference)	
Seroma	44	4	1.92 (0.63 – 5.82)	0.250
Infection	5	2	12.78 (2.04 – 80.30	0.007
Bleeding	8	1	2.74 (0.32 – 23.21)	0.355
Skin necrosis	7	1	3.20 (0.37 – 27.66)	0.291
≥ 2 complications	14	2	3.20 (0.68 – 15.13)	0.143
Missing	0	0		
Previous revision surgery performed				
No	395	22	1.00 (reference)	
Yes	147	11	1.37 (0.65 – 2.90)	0.409
Missing	*33*	*0*		
BMI·				
Normal (18.5 – 30)	438	18	1.00 (reference)	
Underweight (<18.5)	8	1	3.33 (0.39 – 28.55)	0.272
Overweight (>30)	23	3	3.50 (0.95 – 12-87)	0.059
Missing	*73*	*11*		
Smoking·				
Never smoked	383	19	1.00 (reference)	
Currently smoking	65	11	3.90 (1.76 – 8.65)	0.001
Former smoker	73	2	0.54 (0.12 – 2.37))	0.414
Missing	*21*	*1*		
Immunosuppressive treatment·				
No	498	31	1.00 (reference)	
Yes	9	2	3.57 (0.74 – 17.24)	0.113
Missing	*35*	*0*		
Antihypertensive medication·				
No	454	26	1.00 (reference)	
Yes	42	5	2.08 (0.76 – 5.70)	0.155
Missing	*46*	*2*		
Diabetes·				
No	502	31	1.00 (reference)	
Yes, with medication^^	6	2	5.40 (1.05 – 27.85)	0.044
Missing	*34*	*0*		

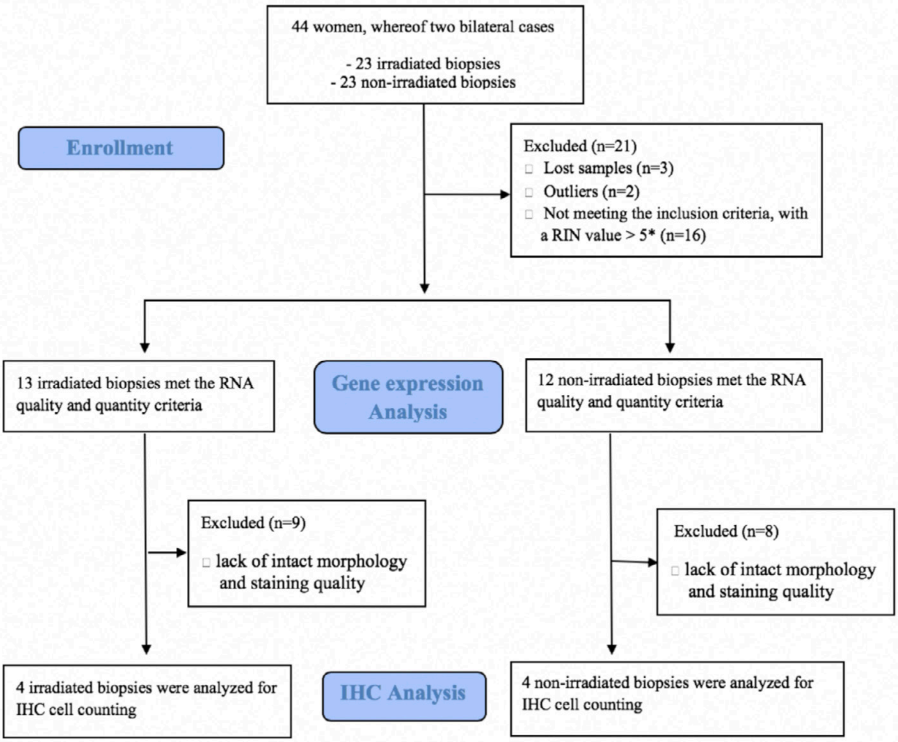
*final expander volume or fixed-volume implant size. **reporting only patients who had received RT after IBR. ^ confirmed by positive bacterial cultures and/or elevated C-reactive protein. ^^including oral medication and/or insulin; ·registered at the time of IBR, not at implant revision surgery

Both node positivity and axillary node dissection increased the risk of implant failure five-fold. Both prior RT and PMRT were a significant risk factor for implant failure. However, data on RT fields showed that this increased risk was only seen in patients with locoregional radiotherapy and not if it only targeted the chest wall. To differ between the effect of locoregional radiotherapy and that of axillary surgery, both covariates were entered into the same regression model. Interestingly, locoregional radiotherapy lost its significance (OR 2.11, 95% CI 0.85-5.24) when compared with no radiotherapy, while axillary lymph node dissection retained its significant negative effect on the risk of implant removal (OR 2.99, 95% CI 1.08-8.27).

4.3 Study V

4.3.1 Human tissue specimens

A prospective cohort of 44 patients was available for biopsies, with two patients having biopsies from both breasts. Biopsies from 25 breasts met the criteria set for RNA quality and quantity and were included in gene expression analysis, see **Figure 2** (corresponding to Figure 1 in study V). From completion of RT to capsular biopsy, median time was 35 (18-304) months. The median radiation dose was 50 (46-50) Gy.



Flow chart for the final cohort of biopsies included in the gene expression analysis; 13 irradiated- and 12 non-irradiated biopsies and the immunohistochemistry analysis; 4 irradiated- and 4 non-irradiated biopsies. *RIN, RNA integrity number, is an algorithm for assigning integrity values to RNA measurements.

Figure 2. Flowchart of the cohort of biopsies.

4.3.2 RNA Extraction

Of the 25 biopsies selected for gene expression analysis, 13 came from irradiated breasts and 12 from non-irradiated breasts, with a RNA integrity number greater than 5 and with a quantity of more than 100ng.

4.3.3 Gene expression

Microarray experiments were conducted at two time points and therefore subjected to a batch-control analysis showing a negligible temporal effect, which further validated the reproducibility of the experiment. Altogether 3422 transcripts, registered as encoded genes according to Entrez Gene, were present in at least one of the groups. A set of the most differentially expressed transcripts, with a raw p-value below 0.05, were selected for enrichment testing (n=227). Gene ontology (GO) analysis showed that the selected radiation-responsive genes were mainly involved in inflammatory response among the top 21 identified GO biological processes ($p<0.05$; FDR<5%). Both innate and adaptive immune responses were represented. The top three GO biological processes identified were humoral immune response mediated by immunoglobulins, followed by complement activation and B cell-mediated immunity (Table 1 in study V). In the Reactome gene sets analysis, scavenging of heme from plasma, C2 and C4 activators and binding/uptake of ligands by scavenger receptors were the most dysregulated (Table 2 in study V).

4.3.4 Estimating the composition of immune cells

CIBERSORT was used to estimate the immune cell composition of the 25 samples and to quantify the relative levels of different cell types in a mixed cell population. Macrophages and T-cells were the most common immune cells in the biopsy material, see **Figure 3** (corresponding to Figure 2 in study V). No significant differences between irradiated and non-irradiated samples were seen regarding the numbers of macrophages and T-cells, except for γ/δ T cells, which were more common in irradiated biopsies ($p=0.022$).

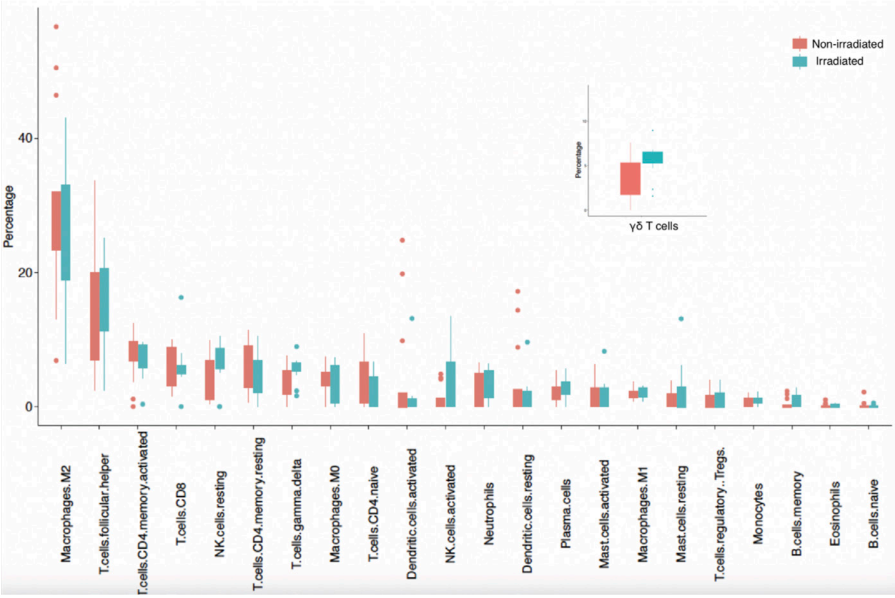


Figure 3. *Immune cell composition*

Macrophages and T-cells were most common. There were no significant differences between the RT- and RT+ groups, except for γ/δ T cells (p=0.022).

4.3.5 Immunohistochemistry

The most relevant cell types according to enrichment testing were analysed with cell-specific markers. There were only eight corresponding biopsies with intact morphology of the capsular surface and adequate staining quality for cell counting out of the 25 biopsies that were included in gene expression analysis. Consentient cell counts were obtained by two blinded evaluators. There was a significantly higher number of CD68+ cells compared to CD3+ cells (p=0.0062) and CD20+ cells (p=0.0025) in the total number of analysed stainings, corresponding to CIBERSORT results. No differences were seen between RT+ or RT- biopsies except for CD20+ cells that were more commonly seen in the RT+ biopsies (p=0.016), see **Figure 4** (corresponding to Figure 3 in Study V). There was evidence of B-cell infiltration in irradiated biopsies only.

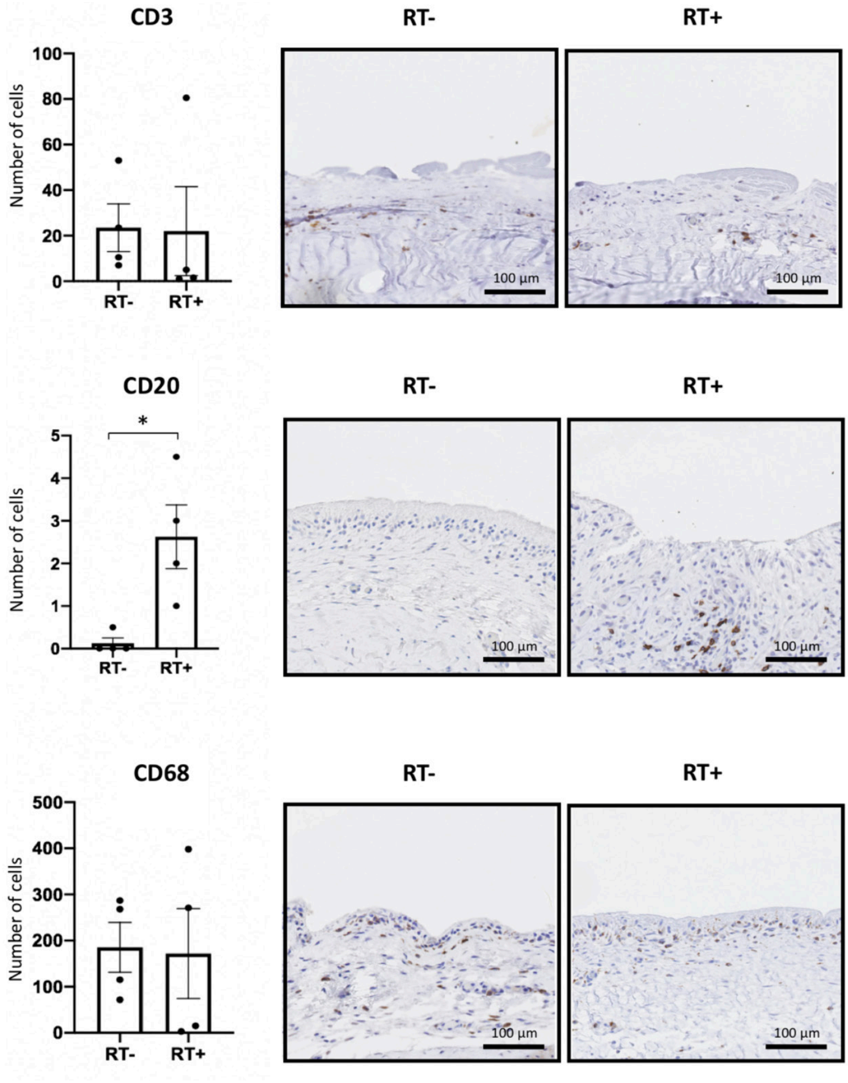


Figure 4. *Results of the immunohistochemistry*

Staining of capsular biopsies for T-cells (CD3+), B-cells (CD20+) and macrophages (CD68+) revealed that CD68+ cells were significantly more prevalent than CD3+ and CD20+ cells ($p=0.0062$ and $p=0.0025$ respectively, for RT- and RT+ groups combined). Comparison of staining in irradiated and non-irradiated groups identified differences for CD20+ cells, with a significantly higher frequency in the irradiated material ($p=0.016$). Indeed, B-cell infiltration was only evident in the irradiated biopsies.

5 DISCUSSION

In this PhD thesis we have investigated the causes of variations in BCS and IBR rates in the six Swedish healthcare regions, which could not be explained by differences in tumour characteristics or age distribution. Instead, a significant disparity in patient information and involvement in decision-making was found, congruent with differences in both BCS and IBR rates. This remained true when selecting groups of women without any contraindications against IBR or BCS according to available data. Socioeconomic factors were also significantly associated with both BCS and IBR rates, but did not eliminate the strong significance of patient information and involvement. Since responders to the patient survey differed from non-responders, results cannot be generalized; on the other hand, however, the observed disparities would have been even more significant with a higher response rate since the characteristics of non-responders were much similar to those of women not receiving IBR and reporting not being well informed.

Furthermore, we evaluated the optimal timing and clinical risk factors for complications after implant-related revision surgery following a previous IBR, with special regard to PMRT. Even though RT significantly increased the risk of implant failure following implant exchange surgery, we could not find any support of optimal timing of the exchange procedure in relation to PMRT, which is potentially due to the fact that a minimum time frame of six months past PMRT is regarded gold standard in Stockholm since many years. Interestingly, previous axillary lymph node dissection and a history of a previous postoperative infection emerged as important risk factors to take into consideration. Finally, we compared gene expression levels and immune cell distribution between irradiated and non-irradiated capsular biopsies, and could observe a sustained innate and adaptive immune response.

In the preoperative meeting between the patient and the responsible breast surgeon and the supporting breast care team counselling for the upcoming treatment, there are several factors to take into consideration. First of all, tumour characteristics: in our study, even though tumour parameters varied between the healthcare regions, these factors could not explain regional variations in either BCS or IBR rates as they did not follow the same pattern. Since the counselling breast surgeon does not know the full extent of the disease at this preoperative meeting, only factors commonly available at this time were considered. Tumour characteristics, however, can already at this time give some hints about appropriate adjuvant therapies such as radiotherapy. Secondly, patient factors: unfortunately, common risk factors associated with inferior IBR outcome, such as smoking and obesity, were not available in our early analyses. Likewise, the size and shape of the breast plays a major role during preoperative counselling but was not registered in this retrospective study. With the help of the patient questionnaire, we could at least assess the most important patient factors, i.e. patient preference, perceived information and feeling of being involved in the decision. Thirdly, the breast care team: not only the surgeon is responsible for correct information and counselling, but this task is shared between the surgeon, the members of the multidiscplinary team conference and the breast nurses who commonly have more frequent contact with the patient than the surgeon. Even in clinics where plastic surgeons perform or assist the IBR, information regarding reconstructive alternatives will typically be delivered by the breast surgeon.

Hence, a large part of preoperative patient information – even though shared with and supported by breast nurses – lies with the breast surgeon. This raises the question whether surgeons and their teams trained in and familiar with reconstructive methods are more likely to inform patients about their reconstructive options. The fact that rates of preoperative patient information and involvement regarding IBR were highest in the Stockholm region, where breast and plastic surgeons have performed breast reconstructions independently or jointly since several decades, supports this theory. Accordingly, the same should apply to surgeons and their teams trained in and familiar with breast-conserving oncoplastic techniques, known to increase BCS rates. Again, this hypothesis is supported by the fact that the BCS rate was highest in the Stockholm region (once the IBR group was excluded), were active training and education in oncoplastic surgery has a long tradition. The lack of plastic surgery services in non-university hospitals had a significant impact on IBR rates as well as on patient information. This problem could be confronted by employing plastic surgeons at or tying them to non-university hospitals on a consultancy basis, have more surgical training in oncoplastic and reconstructive techniques for breast surgeons, and increase collaboration between breast and plastic surgeons (87).

Some argue that patients with relative risk factors, i.e. factors such as PMRT, decreasing the chances for a fully satisfying IBR but not regarded as clear contraindications, should not receive full access to and hence information on IBR options (88). Several studies describe the undeniable negative effects of PMRT on the surgical and cosmetic outcome after IBR (89,90); few reports, however, focus on the patients' perspective. Is it important to remember that when presented with the choice of IBR or DBR versus no reconstruction, even after being informed of potentially negative effects of PMRT, the majority of women still chose IBR (91,92), would choose it again (93), and would recommend it to others (70). This should encourage the surgeon to openly discuss advantages and disadvantages of each option suitable for the individual patients with her specific preconditions, disease characteristics and preferences, and thereby help the patient to make an own informed, evidence-based decision.

When a patient perceives a lack of information about treatment options, this may be due to contributing factors from the informing part (surgeon and other members of the breast care team), the receiving part (the patient, her family and friends), or from both. In modern times, the patient-surgeon relationship has developed more into a shared decision-making and informed consumerist model, where women who take a more active role report a higher postoperative satisfaction (65). This type of information-seeking behaviour is more common among women with a higher level of education (94). Patient participation in the surgical decision-making process increases postoperative quality of life and patient satisfaction compared with a more paternalistic (surgeon-based) decision-making model (58). Importantly, this holds true also for the elderly, a subgroup with equally satisfying IBR results as in younger patients, who are however less likely to be offered any type of reconstruction (95).

When taking a decision for BCS, the travel distance to the nearest hospital may play a significant role since adjuvant radiotherapy is a part of the concept of breast conservation (96,97). Living at a longer distance from a RT department is associated with lower BCS rates (98). The RT treatment usually requires 3-5 weeks of daily visits, and the effort of daily travel or prolonged overnight stays may affect the choice of surgical procedure (99). In our study, however, the North region with the longest distances to the next hospital had the second highest BCS rate, and a high rate of preoperative information regarding BCS.

BCS rates were significantly affected by socioeconomic background, even after adjusting for other patient and tumour characteristics. This is in line with previous findings showing the link between higher socioeconomic status and increased BCS rates (62,63). The fact that women with a lower socioeconomic status had a higher tumour stage has previously been shown (97) and was here confirmed, likely influencing surgical choices, too. Patients receiving IBR were socioeconomically stronger than all other groups. Commonly, such patients are also less likely to be obese, smoke, or have other comorbidities, which would be contraindications for an IBR and thus a reason not to inform the patient about reconstruction more in detail (58,94,100,101). Unfortunately, such data were not available in the present study.

In other questionnaire-based studies, a higher non-response rate has been seen in groups with lower socioeconomic status (102). In an American cohort with different types of breast reconstructions, there was a significantly higher non-response rate among women of non-white race and low household income (103). In our cohort, a majority of the responders had a higher socioeconomic status, which decreases the external validity of the results. The inequality in received information and involvement would thus, however, have been even more significant if more of the non-responders had completed the questionnaire. Results suggest that women with a lower socioeconomic status receive less information or perceive having received no or less information; thus, such information should probably be better adapted to educational level and health literacy. Even though this demands more flexibility in the interaction between the patient and breast care team, it is essential to allow the patient to feel involved in the decision-making process (65). If women with a lower socioeconomic background do not feel informed about their surgical options or supported in their decision, these barriers need to be identified and additional support strategies established. For example, standardized information strategies in oral, written and visual form are known to improve knowledge (104) of surgical treatment options and increases satisfaction with decision-making (105). Take-home information booklets and online resources for patients to read when feeling less distressed might also increase their understanding of available options (106). Further measures, such as assigning a dedicated contact nurse and creating national information leaflets, have been introduced in Sweden after the evaluation of our cohort, and a follow-up study would therefore be of significant interest.

In an immediate two-stage breast reconstruction using tissue expanders, the optimal timing of the exchange procedure to a permanent implant (if deemed necessary) after completion of PMRT is still debated and mostly based on clinical judgement. Exchange can also take place before PMRT without any documented difference in complication rates (107). Women not receiving PMRT may exchange their expander as early as one month after completed expansion (73,108). The recommended timeline from completion of RT to exchange procedure ranges from three to six months (109,110), with a potential benefit in waiting at least six months: according to Peled et al, (109) the implant failure rate after implant exchange three versus six months after completed RT was 22.4% versus 7.7% (p=0.036). In Sweden, the expander-implant exchange procedure is traditionally planned at least six months after the radiotherapy completion, which is probably the reason why our results could not confirm an effect of time.

Nodal treatment comprises both lymph node surgery and locoregional RT in patients diagnosed with axillary macrometastases in Sweden, which significantly increased the risk of implant failure in our study. Scarring and fibrotic changes after axillary treatment may disturb the lymphatic drainage from the chest wall, leading to increased sensitivity to infection and prolonged wound healing (111). Axillary surgery has previously been proven to have a stronger association with long-term negative effects such as arm lymphedema than locoregional RT (20), an observation which was confirmed by our data on implant failure.

Unexpectedly, our results showed that even a transitory infection after the initial IBR was a strong predictor for implant failure after the subsequent exchange procedure. A plausible explanation for this could be a lingering subclinical infection, in spite of a clinically successful antibiotic treatment, causing capsular contraction due to a presence of re-activated bacteria in the capsular tissue (112). Unfortunately, we did not have any information on signs of infection after the revision surgery, nor were routine tissue cultures from implant capsule taken, which could have reinforced this hypothesis.

Of all of clinical risk factors identified in study IV, RT, affecting both the adjacent muscle and subcutaneous tissues, remained the strongest risk factor for capsular contracture. At the interface between the implant and surrounding tissues, there is a natural foreign body reaction that contributes to capsular formation by means of inflammatory cell infiltration, as demonstrated in study V. High frequencies of macrophages and T-cells were identified in capsular biopsies, regardless of radiotherapy exposure, while B-cell mediated immunity and humoral immune response seemed predominantly linked to radiotherapy in the gene expression analysis. This was further supported by a significantly more pronounced B-cell infiltration in irradiated tissues as assessed by immunohistochemistry. This may support the hypothesis that RT can potentiate the foreign body reaction by means of an adaptive immune response. B-cells act as antigen-presenting cells for the activation of e.g. T-helper cells, which means that more CD40 ligands are expressed on the T-helper cells and more cytokines are produced. Theoretically, this could serve as an explanation why RT perpetuates a chronic inflammatory response. A chronic inflammatory response eventually leads to fibrosis and impaired tissue vascularity (66). This type of late adverse effect caused by RT may further explain why previously administered RT has been identified as a strong risk factor for implant surgery complications (70). In those cases, the irradiated tissue may need to be replaced by non-irradiated tissue transferred from other parts of the body as an autologous reconstruction, e.g. a DIEP flap. However, in study IV, we excluded implants removed due to conversion to autologous reconstructions since the underlying reasons of implant removal was not only related to surgical complications, but sometimes related to the patient's wish or an inferior cosmetic result which is often related to capsular contracture. Further investigation of infiltration of immune cells around breast implants, with or without RT, could contribute to the development of therapeutic strategies to attenuate capsular formation.

Strengths and limitations

Study I-III

There is always a risk of recall bias in any retrospective study design (113), which should, however, have been equally distributed over the compared groups. Further, no data on risk factors such as comorbidity, high BMI and smoking were available, which might nevertheless have influenced the surgical decision-making process. Since our results stem from 2013, they could be viewed as outdated considering that BCS and IBR rates have significantly improved since we conducted our studies; subsequent national reports, however, continue to show regional differences despite an increased national IBR rate (4). The main strengths of studies I-III are the high coverage and validity of two national registers containing detailed clinical and socioeconomic information, as well as the fact that patient perspectives were integrated into analyses with a high response rate (85,86). Moreover, the impact of socioeconomic background is especially interesting in a country with tax-funded healthcare guaranteeing equal care to all citizens, since the influence of reimbursement should be negligible as a confounder.

Study IV

In most retrospective studies based on medical chart reviews, there is a risk of limited information since the viewer can only register data that have been recorded by others without any standardized data requirements. This, however, was not true for the main outcome since reoperations are always registered in the medical charts by means of standardized codes. Even though our studied cohort was large, there was a surprisingly low number of events, which rendered advanced statistical analyses inappropriate. Furthermore, overall implant removal rates would have been higher if we had included implant removal due to discomfort or inferior cosmetic results, often resulting in a conversion to an autologous reconstruction; this was, however, not the aim of our evaluation. It would be an interesting future addition to our study to also evaluate such conversion rates since these should most likely vary with the receipt of RT, too.

Study V

Due to the rather small sample size, gene expression results need to be interpreted with some caution. The overall RNA quality was low, which shows the difficulties of preparing sensitive tissues in a clinical environment. The lack of inter-individual differences may be due to no paired cases with a non-irradiated control. Paired synchronized samples of irradiated biopsies and non-irradiated internal controls could have isolated RT effects, which was however not possible in this study.

6 CONCLUSIONS

In summary, we provide explanatory models for variations in BCS and IBR rates in Sweden. Surgical choices were affected by socioeconomic factors, and patient-reported preoperative information and involvement in the decision-making process proved to be strong predictors even after adjusting for other covariates. Our results suggest that socioeconomic background should be taken into account in preoperative counselling, to provide tailored information and equal health care to all individuals.

PMRT was confirmed as a risk factor for implant failure after revision surgery; we could, however, not identify an effect of the time elapsed from completion of PMRT. In addition to known risk factors such as diabetes or smoking, special attention should be paid to previous axillary lymph node dissection and a history of post-IBR infection. Failure rates following revision surgery after IBR, however, are low, so that it appears to be an acceptable option even in the irradiated breast.

For a growing population of women receiving PMRT, it is of paramount importance to better understand the biology behind radiation-induced capsular contracture in order to pave way for future therapeutic and prophylactic strategies. We revealed inflammatory responses in capsular biopsies regardless of RT, while the radiation response specifically involved B-cells. We encourage future search for therapeutic treatment strategies to attenuate capsular contracture and its severe clinical consequences in patients undergoing implant-based breast reconstruction.

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